THE EFFECT OF ENFLURANE AND FENTANYL ANAESTHESIA ON HUMAN PLATELET AGGREGATION *IN VIVO*

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ABSTRACT

In 30 patients undergoing major operations, and anaesthetized with either nitrous oxide, oxygen and enflurane, or nitrous oxide, oxygen and fentanyl, there was no significant alteration in platelet aggregation induced by either adenosine diphosphate or collagen. The absence of any significant effect on platelet function was confirmed by an unchanging thromboelastogram pattern during the study.

In 1971 Ueda demonstrated a dose-dependent decrease in platelet aggregation with the *in vitro* mixing of anaesthetic gases and dog blood.¹ Attempts to demonstrate *in vivo* depression of platelet function in man during anaesthesia and operation have produced equivocal results.^{2,3,4} It was the purpose of this study to assess alterations in platelet aggregation and function in man during the administration of nitrous oxide, oxygen and enflurane anaesthesia and nitrous oxide, oxygen and fentanyl anaesthesia, using the platelet aggregometer and thromboelastograph.

METHODS

Thirty adult patients undergoing major operations and known not to be taking antiplatelet drugs or anticoagulants were randomly selected to receive either nitrous oxide, oxygen and enflurane, or nitrous oxide, oxygen and fentanyl anaesthesia. Preoperative platelet counts, prothrombin time and activated partial thromboplastin time were normal in all patients. Only one patient in the study had received anti-cancer chemotherapy, but none in the past year. All were evenly matched as to age, sex and type of operation (see Tables I, II). The patients were premedicated with morphine sulphate (5–10 mg, pentobarbitone sodium 50–100 mg and glycopyrrolate 0.2–0.4 mg or atropine 0.4–0.6 mg. All drugs were administered intramuscularly, ap-

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TABLE I
PATIENT DISTRIBUTION BY AGE, SEX

	Male	Female	Age	Mean
Enflurane	10	5	38–71	58
Fentanyl	13	2	20–67	57

TABLE II
TYPE OF OPERATION

	Enflurane	Fentanyl
Bowel resection	0	3
Head/Neck	5	1
Urinary tract	2	4
Superficial	1	0
Thoracotomy	7	7
	15	15

proximately one hour before induction of anaesthesia, the dose being determined by the patient's state of anxiety, height and weight. On arrival in the operating room a 16-gauge central venous pressure line was inserted and all blood specimens were removed through this catheter. In twenty patients (ten fentanyl, ten enflurane) blood was drawn before induction of anaesthesia (control), one hour after start of the operation and in the recovery room within one hour of the end of anaesthesia. Ten of the study patients (five fentanyl, five enflurane) underwent operations which were either preceded by flexible bronchoscopy through the tracheal tube, or by at least one-half hour of preoperative preparation under anaesthesia. This allowed time for drawing samples before anaesthesia, during anaesthesia but before the operation, during the operation and in the immediate postoperative period. Thus, if anaesthesia were to induce changes in platelet

function, opposite in character to those induced by operation *per se*, this would be evident.

The first 10 ml of all blood samples were discarded. Drawn blood was then mixed nine parts to one part 0.11 M sodium citrate. Platelet-rich plasma was prepared by centrifuging whole blood at $270 \times g$ for ten minutes and removing 2.5 ml of the supernatant. Platelet poor plasma was obtained by further centrifuging the remaining sample at $3000 \times g$ for twenty minutes. Platelets were maintained at room temperature in capped plastic tubes 1–1.5 hours before being placed into the aggregometer cuvettes and warmed to $37^\circ C$ for two minutes before testing.

Platelet aggregation was determined using Born's turbidimetric technique which measures alterations in optical density in platelet rich plasma as an index of the number of platelets in suspension.^{5,6} Aggregation decreases the number of platelets in suspension, and thus decreases optical density.

The samples were tested in a PAP-2 aggregometer (Bio-Data Corp., Willow Grove, Pennsylvania, USA), using adenosine diphosphate (ADP) at 2×10^{-5} M concentration, and collagen 0.26 mg/ml concentration. Aggregation curves, using adjusted platelet rich plasma of 250–300 mm³, were interpreted by measuring the maximum decrease in optical density. Each patient's pre-anaesthetic curves were used as control.

Platelet counts were done on all blood specimens, using a phase microscope.

For thromboelastography⁷ 0.3 ml of untreated whole blood was pipetted into the rotating cuvette of the thromboelastograph (Hellige Co., Glenview, Illinois, USA), which had been pre-warmed for at least 30 minutes. The piston was then lowered into the sample, covered with mineral oil and clot formation was measured graphically. The forming clot binds this piston to the rotating cup. Thus the time to beginning clot formation, the clot formation time itself and the tensile strength of the clot can all be measured. Alterations in platelet function (as with any other component of the clotting process), would then be demonstrated as an altered thromboelastogram, when compared to control.

Anaesthesia was induced in all patients with intravenous thiopentone $3\text{--}4 \text{ mg} \cdot \text{kg}^{-1}$ and tracheal intubation was effected after $1\text{--}2 \text{ mg} \cdot \text{kg}^{-1}$ of succinylcholine chloride administered intravenously. Pancuronium bromide was used as needed for relaxation during operation and was reversed at the end of the procedure.

Anaesthesia was maintained with 60 per cent nitrous oxide with oxygen, with enflurane 0.5–2.0 per cent or intermittent intravenous doses of fentanyl as judged necessary. Intravenous fluids consisted of 2.5 per cent dextrose with 0.45 per cent saline $5 \text{ ml} \cdot \text{kg}^{-1}/\text{hour}$ with whole blood and fresh frozen plasma as needed during the operative procedure.

Two patients received whole blood before the drawing of the intraoperative blood specimen, and the average amount of blood received by these two patients was 300 ml. Four patients had received fresh frozen plasma, to an average 150 ml.

The data were analyzed using Student's t-test.

RESULTS

With one exception, all control platelet specimens were aggregated by the adenosine diphosphate solution, and this response was not altered by anaesthesia or operation. Similarly, all control specimens were aggregated by collagen, with no significant alteration in the pattern of aggregation during the study period.

Although the average platelet aggregation response to collagen did decrease slightly after induction of anaesthesia, and before the operation, the change is not statistically significant for either the enflurane or the fentanyl group. Actually, while the mean aggregation did decrease, decreased aggregation occurred in two of five patients receiving enflurane, while in three, aggregation actually increased. Similarly, in the fentanyl group aggregation decreased in two, increased in one and remained unchanged in two patients.

Platelet aggregation increased after beginning the operation, but again the changes were insignificant and unpredictable (See Tables III, IV).

The platelet counts in all patients remained unchanged, except for slight dilutional decreases associated with the administration of large amounts of whole blood or fresh frozen plasma. This slight difference did not alter the study results.

Thromboelastography confirmed the absence of any change in clot formation or the development of clot tensile strength.

DISCUSSION

Although Ueda's work demonstrated decreased platelet aggregation, it is difficult to as-

TABLE III
MEAN PER CENT PLATELET AGGREGATION WITH ADENOSINE DIPHOSPHATE
($\pm$ Standard Deviation)

Agent	Preoperative	Anaesthesia without operation	Intraoperative	Postoperative
Fentanyl	79.6 ($\pm$ 9.7)	76 ($\pm$ 4.2)	82.5 ($\pm$ 9.2)	82.1 ($\pm$ 9.6)
Enflurane	77.7 ($\pm$ 8.8)	77.0 ($\pm$ 7.6)	85.3 ($\pm$ 9.7)	83.0 ($\pm$ 10.9)

TABLE IV
MEAN PER CENT PLATELET AGGREGATION WITH COLLAGEN
($\pm$ Standard Deviation)

Agent	Preoperative	Anaesthesia without operation	Intraoperative	Postoperative
Fentanyl	84.3 ($\pm$ 7.5)	76 ($\pm$ 10.8)	85.3 ($\pm$ 7.9)	87.3 ($\pm$ 5.0)
Enflurane	83.0 ($\pm$ 8.4)	76.4 ($\pm$ 5.5)	85.3 ($\pm$ 9.1)	85.0 ($\pm$ 7.6)

sess the significance of this in man. *In vitro* platelet studies are of questionable reliability. Since platelets are notoriously labile. Similarly, species differences among various study animals make it impossible to extrapolate animal data and to consider it applicable to man.^{8,9}

Although all patients had cancer, the results of the study are not vitiated, since it has been demonstrated that platelet aggregation induced by adenosine diphosphate is no different in patients with cancer than it is in patients with non-malignant disease.¹⁰

O'Brien, Etherington and Jamieson⁴ described a decrease in platelet aggregation in response to both adenosine diphosphate and collagen during thoracotomy. This decreased aggregation was short-lived, returning to normal on the first post-operative day. Although the anaesthetic agents and techniques were not specifically mentioned, it is stated that blood was selected and tested after administration of each anaesthetic agent, including meperidine, thiopentone, curare, neostigmine *et al.*, and that the anaesthetic agents *per se* apparently had no effect on aggregation. Thus, the decreased platelet aggregation was attributed to release of adenosine diphosphate induced by operation, without further evidence.

Our study, on the contrary, indicates no decrease in platelet aggregation attributable either to anaesthesia or operation.

Platelet aggregation may be thought of as occurring in two stages.^{9,11} Stage I is induced by the addition of adenosine diphosphate, which causes platelets to become sticky, to adhere to each other, to aggregate and precipitate. Stage II may

be induced by the addition to platelet rich plasma of collagen, a weaker solution of adenosine diphosphate or epinephrine^{8,9,11-13} causing the release of endogenous adenosine diphosphate from within the platelet, thus increasing the periplatelet concentration of adenosine diphosphate, platelet adhesiveness and aggregation. Our study thus indicates that neither the first stage of platelet aggregation nor the second is altered by enflurane or fentanyl anaesthesia.

Bleeding time determinations were not done because of the lack of correlation between alterations in adenosine diphosphate or collagen induced platelet aggregation and bleeding time.¹⁴

It has been postulated that a non-specific membrane stabilizing effect of such drugs as chlorpromazine and imipramine may account for their ability to inhibit platelet aggregation, and by extension this membrane stabilizing effect may account for the central nervous system effects of these drugs.¹⁵ Local anaesthetics have also been shown to inhibit platelet aggregation induced by adenosine diphosphate,¹⁶ presumably by altering mechanisms of calcium transport and utilization in the nerve. However, we see no indication that general anaesthetics share this membrane effect in platelets and thus suggest that the concept of membrane stabilization cannot logically flow from an effect of anaesthetics on platelet function.

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RÉSUMÉ

Lors d'interventions majeures subies par 30 patients anesthésiés au protoxyde-oxygène fentanyl ou protoxyde-oxygène enflurane, il n'y a pas eu d'altérations significatives de l'aggrégation plaquettaire induite par l'adénosine diphosphate ou le collagène. L'absence d'effet sur la fonction plaquettaire a été confirmée pendant cette étude par la stabilité du thromboélastogramme.